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Research Article

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Screening of carbon sources/prebiotics and amino acids in the medium for *Streptococcus thermophilus* using Plackett–Burman design

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ABSTRACT

Previous studies have shown that the carbon sources/prebiotics and amino acids have an important influence on the growth of Streptococcus thermophilus. Out of consideration of the optimal substances for growth of Streptococcus thermophilus, viable counts was studied in the medium containing various carbon sources/prebiotics (glucose, sucrose, lactose, fructose, maltose, xylooligosaccharides, fructooligosaccharides, galactooligosaccharides, isomaltooligosaccharide, stachyose, inulin) and amino acids (glutamate, glycine, serine, leucine, valine, lysine, threonine, phenylalanine, arginine, aspartic and Vc). The results indicated that xylooligosaccharides, lactose and isomaltooligosaccharide out of selected carbon sources/prebiotics affect the growth of Streptococcus thermophilus markedly. In addition, these three variables showed all positive effect. Glutamate, lysine and valine in the investigated amino acids showed a significant effect on the growth of Streptococcus thermophilus; moreover, glutamate and lysine had a positive effect on it, but valine negative. Overall, isomaltooligosaccharide and glutamate were the most efficient selected prebiotics and amino acids with positive impact on the growth of Streptococcus thermophilus, respectively.

Keywords: *Streptococcus thermophilus*; the growth media; carbon sources/prebiotics; amino acid

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INTRODUCTION

Dairy starter cultures are of industrial importance for fermented foods [1]. As a major component of dairy starter, *Streptococcus thermophilus* has possessing "Generally Recognized as Safe (GRAS)" status and widely used in the manufacture of dairy products in particular in yoghurt, cheese, dry sausage, salami and sourdough in combination with *Lactobacillus delbrueckii* ssp. *bulgaricus* [2-5]. At present, there have been considerable changes in the preparation of the inoculums. Starter cultures have been used increasingly in concentrated forms for the direct inoculation to the food matrix [6]. Therefore, it is necessary to screen the optimal medium in order to obtain an excellent starter with high viable counts.

Previous studies have shown that M17 media is critical for the growth of *Streptococcus thermophilus*. Therefore, a cheaper media is necessary and the development of economical medium requires selection of carbon, nitrogen, phosphorous, potassium and trace element sources [7-9]. Prebiotics are non-digestible carbon sources that stimulate the growth of beneficial microbial populations [10-11], and the growth rates of *Streptococcus* strains increased when an amino acid mixture was added [12-13].

The Plackett–Burman statistical method offers a design where n variables are studied in $n+1$ experimental runs. These experimental designs are excellent screening methods, as the required number of experimental runs are very few, leading to saving of time, chemicals, glassware and manpower [14-18]. Moreover, the design is orthogonal in nature, implying that the effect of each variable worked out is pure in nature and not confounded with interaction among variables. Experimental design and data analysis using appropriate software makes the analysis easier [7].

In our previous work, nitrogen sources that had an important influence on the growth of *Streptococcus thermophilus* were studied [19]. The purpose of this study was to investigate the influence of various carbon sources/prebiotics and different amino acids on the growth of *S. thermophilus*.

EXPERIMENTAL SECTION

Microorganism and culture conditions The strains *S. thermophilus* were obtained from College of Life Science & Engineering, Shaanxi University of Science & Technology and inoculated three successive times with M17 medium at 42□for 24h until the viability of bacteria stays stable.

Media preparation The basal LAB growth media was used in the study, containing 10g of glucose, 2g of KH_2PO_4, 100 mL of tomato juice(was purchased and made from a local store and kept at 4 ºC prior to use), 0.5ml Tween 80, 7.5g yeast extract, 0.5g peptone, 900mL water. All the media were autoclaved at 118 ºC for 15min.

Screening of carbon sources and amino acids using Plackett–Burman design Plackett–Burman design that comprised 8 factors spanning over 12 runs with each factor fixed at two levels (namely a lower level and a higher level, represent by the +1 and -1, respectively)was used. The ingredients tested are given in Table 1 and Table 2 along with their actual levels including carbon sources (Glucose, Sucrose, Lactose, Fructose, Maltose, Xylooligosaccharides, Fructooligosaccharides, Galactooligosaccharides, Isomaltooligosaccharide, Stachyose, Inulin) and amino acids (Glutamate, Glycine, Serine, Leucine, Valine, Lysine, Threonine, Phenylalanine, Arginine, Aspartic and Vc) which were screened for their significance in viable counts, pH and OD value.

Tab.1 Carbon source/prebiotics at different levels in Plackett–Burman design

Variables	Medium components	Lower level (g/100ml)	Higher level (g/100ml)
X1	Glucose	0.5	1
X2	Sucrose	0.5	1
X3	Lactose	0.5	1
X4	Fructose	0.5	1
X5	Maltose	0.5	1
X6	Xylooligosaccharide	0.5	1
X7	Fructooligosaccharide	0.5	1
X8	Galactooligosaccharide	0.5	1
X9	Isomaltooligosaccharide	0.5	1
X10	Stachyose	0.5	1
X11	Inulin	0.5	1

Tab. 2 Amino acids at different levels in Plackett–Burman design

Variables	Medium components	Lower level (mg/L)	Higher level (mg/L)
X1	Glutamate	5	10
X2	Glycine	5	10
X3	Serine	5	10
X4	Leucine	5	10
X5	Valine	5	10
X6	Lysine	5	10
X7	Threonine	5	10
X8	Phenylalanine	5	10
X9	Arginine	5	10
X10	Aspartic	5	10
X11	Vc	0.4	0.2

Culture conditions 5% active culture was added to each media that were autoclaved after cooling to 50℃, incubated at 42℃, and then pH, OD and viable counts at optional incubation time. The experiments were completed in triplicate and means were calculated for each treatment group at each data collection point.

Analysis Method The pH of culture media directly evaluated through a pH-meter (pHS-3C) at the room temperature and the viable counts was determined according to plate coating method. Morphology of bacteria was measured using Toluidine blue staining method, and OD of the strains measured by Spectrophotometer.

Statistical analysis of the data The statistical analysis performed by the SAS (Version, 12.0) to identify the significant variables and their corresponding coefficients, so that the levels of variables can be managed to obtain a desired output. Hence, the coefficients, sum of squares in percentage (SS %) and confidence interval (CI) were analyzed using the experimental results of the viable bacteria. So the experimental plan, the analysis and the results were obtained.

RESULTS AND DISCUSSION

Effect of carbon sources/prebiotics on growth of *S. thermophilus*

In the present study, the experimental design and results were shown at Table 3. There was no significant difference in pH of these factors, apart from this, OD value which including dead cells did not reflect the number of viable cells. Consequently, the value Y that representing viable counts in the fermentation broth (the unit 10^9 cfu/mL) was used as response value.

Tab.3 The experimental design and results of carbon source/prebiotics Plackett-Burman

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	OD	pH	Y(×10^9cfu/mL)
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	0.826	3.93	0.82
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	0.859	3.91	0.80
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	0.869	3.96	1.13
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	0.869	3.95	1.00
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	0.838	3.98	0.73
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	0.880	3.96	1.02
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	0.851	3.94	1.14
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	0.833	3.94	0.84
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	0.860	3.97	0.97
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	0.886	3.96	1.00
11	-1	1	-1	-1	-1	1	1	1	-1	1	1	0.843	3.97	0.87
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.936	3.98	0.78

As lacking of relevant enzymes, *S. thermophilus* cannot take advantage of macromolecules that we select monosaccharide and oligosaccharides as a carbon source. The experimental analysis shows in Figure 1. Three variables namely xylooligosaccharides, lactose and isomaltooligosaccharide out of 11 variables form higher percentage sum of squares than others, which means that these variables influenced the growth of *S. thermophilus* significantly. Furthermore, the 95% confidence interval of the carbon sources (Fig.2) implying that these three variables showed positive effect. That is, the growth of *S. thermophilus* can be increased when the above three carbon sources were added in medium. Claire *et al*. suggested that the presence of isomaltooligosaccharide could make bifidobacteria a higher proliferation rate [20]. In another study [21], xylooligosaccharides, fructooligosaccharides, galactooligosaccharides and isomalto- oligosaccharide also had positive impact on the growth of *Lactobacillus*. A study on energy sources of yoghurt bacteria showed that lactose was better when used for growth by all the experimental isolates when compared to other sugars [22].

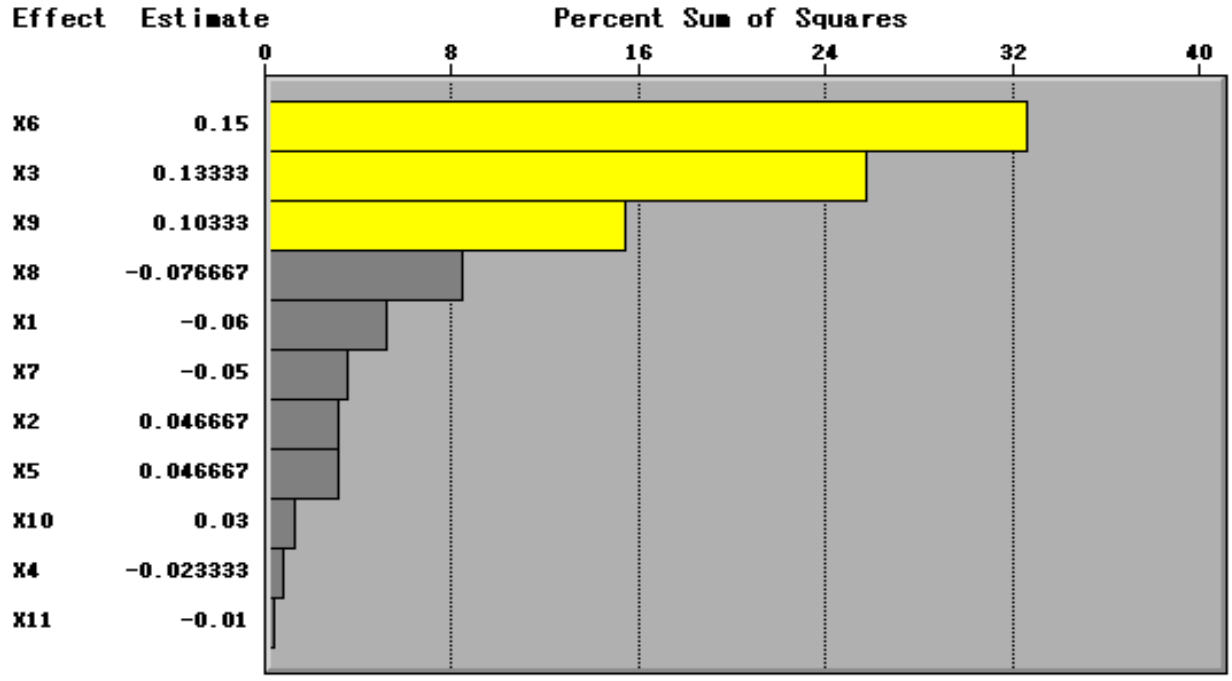

Fig. 1 Main factors figure of carbon source Plackett-Burman

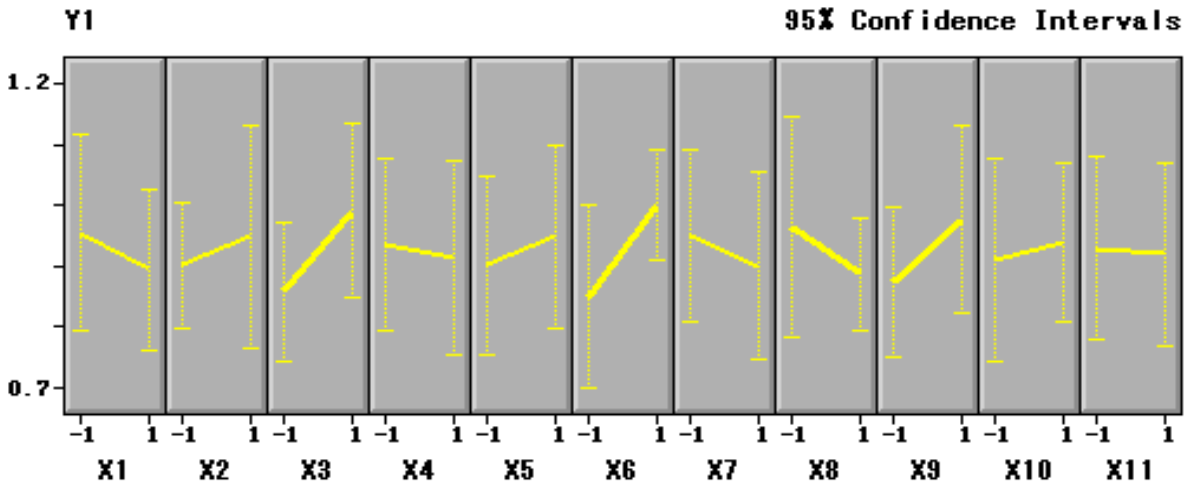

Fig. 2 The 95% confidence interval of carbon source

Effect of amino acids on growth of *S. thermophilus*

The experimental design and results were shown at Table 4.

Tab. 4 The experimental design and results of amino acid Plackett-Burman

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	OD	pH	Y(×10^{9}cfu/mL)
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	0.895	4.29	1.16
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	0.874	4.21	1.28
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	0.901	4.28	0.98
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	0.894	4.28	1.51
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	0.914	4.28	1.15
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	0.900	4.21	1.28
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	0.923	4.19	1.13
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	0.916	4.21	0.82
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	0.943	4.28	1.02
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	0.741	4.19	1.25
11	-1	1	-1	-1	-1	1	1	1	-1	1	1	0.752	4.26	1.38
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.753	4.20	0.88

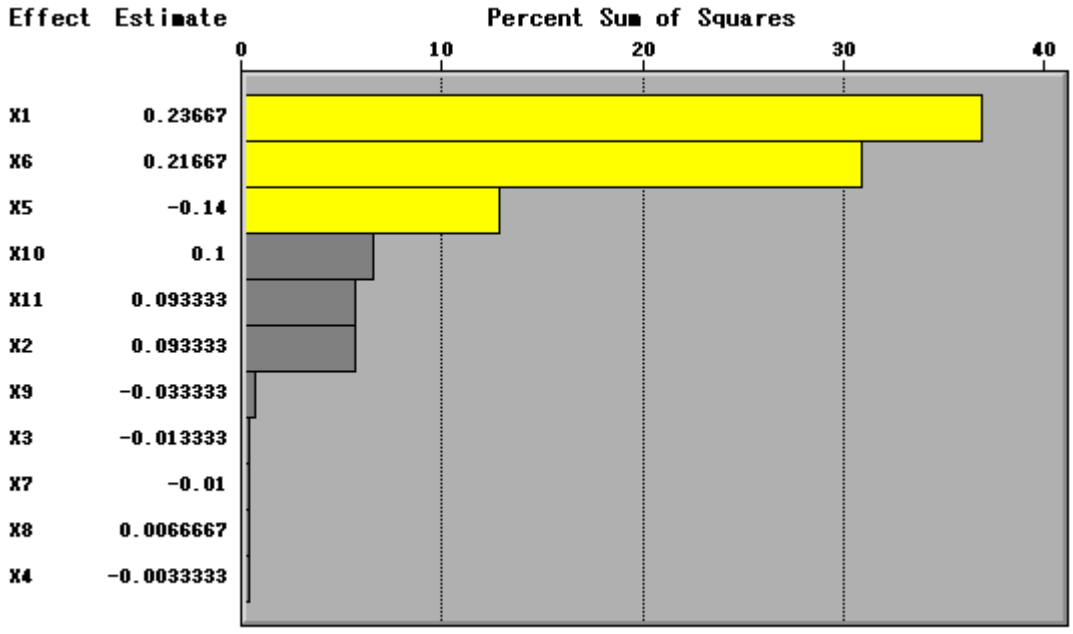

Fig.3 Main factors figure of amino acid Plackett-Burman

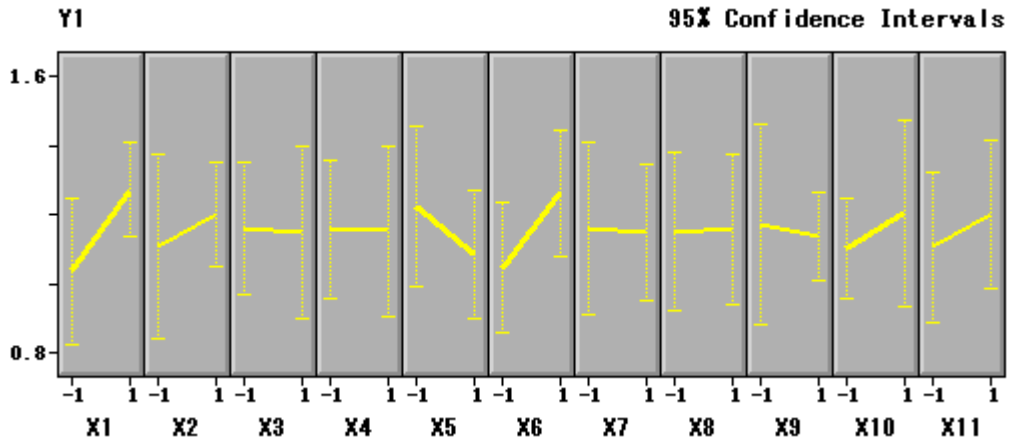

Fig.4 The 95% confidence interval of amino acid

Analysis of the dates for the percent sum of squares revealed presence of glutamate, lysine and valine accounted for a large proportion of it, and it indicated that these three amino acids had a significant impact on the growth of *S. thermophilus* and glutamate was the most significant factor (Figure 3). While the figure that 95% confidence interval of amino acid (Figure 4) agreed with this result. Moreover, glutamate and lysine on the response Y (number of viable cells) showed a positive effect, i.e. the response Y (number of viable cells) increased with the increasing of concentration of these two factors. On the contrary, Valine showed a negative trend. Rapid growth of *Streptococcus thermophilus* mutants in milk could be restored by the addition of four specific amino acids [23], i.e. Glu, His, Met, and Val. A number of essential amino acids (leucine, methionine, glutamate and in some cases phenylalanine) were added and stimulated the growth of *S. cremoris* in milk [12].

CONCLUSION

In conclusion, carbon sources and amino acids have a huge influence on viable bacteria of *S. thermophilus*. Xylooligosaccharides, lactose, isomaltooligosaccharide, glutamate, lysine and valine out of selected substances affect the growth of *S. thermophilus* significantly. In addition, xylooligosaccharides, lactose, isomaltooligosaccharide, glutamate and lysine showed positive effect, but valine had negative trend. Whatever, these positive effectors can be used in the cultivation of *S. thermophilus* and are also conducive to the accumulation of bacterial cell. In addition, the negative trend of valine for growth of *S. thermophilus* suggested that this kind of amino acid must be limited in the medium.Although it has some differ with previous researches; it may be due to species differences and the result that the nutrients stimulate growth of *S. thermophilus* populations gain our interests on its further application.

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